Assessment of different methods to estimate bovine colostrum quality on farm

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vorgelegt von
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<thead>
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<th>Description</th>
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<td>AUC</td>
<td>area under the ROC curve</td>
</tr>
<tr>
<td>FPT</td>
<td>failure of passive transfer</td>
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<tr>
<td>Ig</td>
<td>immunoglobulins</td>
</tr>
<tr>
<td>NPV</td>
<td>negative predictive value</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
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<tr>
<td>RID</td>
<td>radial immunodiffusion</td>
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<tr>
<td>ROC</td>
<td>receiver operating characteristic</td>
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<td>SD</td>
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1 Abstract

1.1 Background

A sufficient intake of ‘good’ quality colostrum provides newborn calves with essential immunoglobulins (Ig) for passive immunity. An Ig concentration > 50 g/L usually indicates colostrum of ‘good’ quality. To determine colostrum quality on farm, an easy to use and accurate method is needed. To date, the most commonly used method on farm is a hydrometer which is inexpensive and provides results fast. Disadvantages such as temperature dependence, lack of sensitivity and partly specificity have been reported. Another method is the use of a Brix refractometer. Previous studies demonstrated that Brix refractometers meet the above-mentioned criteria more accurately than the hydrometer. The reported cut-offs for ‘good’ quality colostrum range from 21 to 23% Brix. The aim of this study was to assess two different hydrometers, an optical and a digital Brix refractometer in terms of accuracy and precision compared with the measurement of IgG by the reference method radial immunodiffusion (RID). Furthermore, this study is the first to provide data on repeatability and reproducibility for Brix refractometers.

1.2 Results

Repeatability and reproducibility for both refractometers were almost perfect with Spearman correlation coefficients ≥ 0.96. Of all 193 examined samples, 34.9% had an IgG concentration ≤ 50 g/L determined by RID. Spearman correlation between the RID and the two hydrometers were 0.65 and 0.49, and 0.62 and 0.68 for the optical and digital Brix refractometer, respectively. Correlation coefficients between Brix refractometers and RID was lower for ‘poor’ (≤ 50 g/L) than for ‘good’ quality samples. Sensitivities varied between 0.90 and 0.96 for all devices. Specificities were
low, ranging between 0.27 and 0.47. Balanced cut-offs were higher for all devices compared with the reference values.

1.3 Conclusion

The digital Brix refractometer is currently the best available method to determine colostrum quality on farm. It is a rapid, convenient, on farm method that provides appropriate classification of colostrum quality, showing best performance at a cut-off of 23.4% in this study. Based on the finding that correlation was lower for ‘poor’ quality samples in this study, it is likely that the level of IgG concentration is associated with the degree of correlation.

1.4 Keywords

Colostrum quality, Hydrometer, Brix refractometer, Validation
2 Background

A sufficient and timely uptake of colostral immunoglobulins (Ig) reduces the risk of disease and neonatal death (QUIGLEY et al., 2001; GODDEN, 2008; FURMAN-FRATCZAK et al., 2011) and is one of the key factors to achieve optimal health for newborn calves. Immunoglobulin concentrations in colostrum vary between cows (SWAN et al., 2007). In general, a concentration > 50 g IgG/L is considered as ‘good’ quality colostrum (KRUSE, 1970; FLEENOR and STOTT, 1980; GODDEN, 2008; WEAVER et al., 2000). An adequate quantity of IgG fed with first colostrum to a calf is 100 to 200 g (MCGUIRK and COLLINS, 2004). A lack of Ig intake by the calf leads to failure of passive transfer (FPT) of Ig (WEAVER et al., 2000). FPT increases morbidity and mortality rates in the first weeks of life and, thus, may cause severe economic losses (MCGUIRK and COLLINS, 2004). Consequently, colostrum quality and its control are of importance in dairy calf rearing. Unfortunately, only few farmers routinely control the quality of colostrum (GODDEN, 2008; VASSEUR et al., 2010; KLEIN-JÖBSTL et al., 2014; STANEK et al., 2014). Those who evaluate colostrum quality, often use inaccurate methods such as visual inspection or weight control (CHIGERWE et al., 2008). To improve colostrum management, an on farm device for quality control needs to be established. On farm devices should be easy to use, rapid, accurate, and inexpensive. Radial immunodiffusion (RID) is considered as the reference method (OYENIYI and HUNTER, 1978; FLEENOR and STOTT, 1980). Because the test is expensive and needs approximately 24 hours to gain results, RID is not suitable for on farm use (OYENIYI and HUNTER, 1978; FLEENOR and STOTT, 1980). A method often used in the field is the use of a hydrometer, which measures the specific gravity of colostrum that correlates with the Ig concentration. Although rapid and inexpensive, it only works accurately within a narrow temperature range and recent research reported low sensitivity and specificity (FLEENOR and STOTT, 1980; MECHOR et al., 1991; MECHOR et al., 1992; PRITCHETT et al., 1994). Another method is the use of an optical or digital Brix refractometer, which measure the refractometric index of liquids on a Brix scale. According to studies under laboratory conditions, Brix measurement reveals high sensitivity and specificity.
compared with the reference method RID (CHIGERWE et al., 2008; BIELMANN et al., 2010; QUIGLEY et al., 2013; VANDEPUTTE et al., 2014; BARTIER et al., 2015). Reported cut-offs for ‘good’ quality, i.e. > 50 g IgG/L, range from 21 to 23 % Brix (BIELMANN et al., 2010; QUIGLEY et al., 2013; BARTIER et al., 2015). The aims of our study were to evaluate the accuracy and precision of four different devices (two hydrometers, an optical and digital Brix refractometer) to determine IgG concentrations in bovine colostrum on farm. Furthermore, this study is the first to provide data on repeatability and reproducibility for both kinds of Brix refractometers.
3 Methods

3.1 Pre-study

To evaluate inter-rater reliability of the optical Brix refractometer (Handheld Refractometer MHRB-40 ATC, Mueller Optronic, Erfurt, Germany), 150 first colostrum samples, taken in a previous study, were tested by two persons independently. Furthermore, these samples were measured two times with the optical and digital Brix refractometer (Digital Refractometer HM-DREF-1, Hebesberger Messtechnik, Neuhofen, Austria) by the same person to evaluate intra-rater reliability. First colostrum samples used were taken immediately after milking within 1 hour after parturition from the milk bucket and stored at -20°C. All samples were thawed at room temperature and homogenized before testing.

3.2 Sampling

First colostrum samples of 195 Holstein Friesian cows were collected on one large commercial dairy farm in Slovakia between June 2013 and October 2014. Samples were taken from cows in second or greater lactation. Colostrum of first lactating cows could not be sampled as heifers were housed on a separate premise of the farm. First colostrum was machine milked in an individual bucket by the farm personnel within one hour after parturition. Study samples were taken immediately after milking from the bucket. Samples were stored into 250 ml sanitized plastic tubes (Wide mouth leak proof plastic bottles, Kautex Textron, Bonn, Germany) and either immediately tested or frozen (-20°C). Frozen samples were thawed in warm water baths (approximately 40°C) to be analysed likewise as the fresh samples. For an accurate measurement each sample was thoroughly inverted by hand before testing. Furthermore, two 1.5 ml aliquots of the fresh samples were frozen (Safe seal tubes, Eppendorf, Hamburg, Germany) for RID testing in the laboratory (Institute for Milk Hygiene, University of Veterinary Medicine, Vienna, Austria). Cow identification number, collection date, time of birth and volume of first colostrum was recorded.
3.3 Devices

Samples were analysed by two hydrometers with different temperature optima. According to the manufacturers` instruction, hydrometer 1 (Colostrum densimeter, Kruuse, Langeskov, Denmark) has its optimum at 20°C and hydrometer 2 (Colostrometer®, Pfizer Animal Health Germany GmbH, Berlin, Germany) at 37°C. To maintain the desired temperature, the samples were either cooled or warmed in water baths. Temperature was controlled with a universal thermometer for liquids (TFA-Dostmann, Wertheim-Reicholzheim, Germany). Additionally, all samples were measured with an optical and digital Brix refractometer. The scale for the optical Brix refractometer (Handheld Refractometer MHRB-40 ATC, Mueller Optronic, Erfurt, Germany) ranged from 0 to 40 % Brix. The scale of the digital Brix refractometer (Digital Refractometer HM-DREF-1, Hebesberger Messtechnik, Neuhofen, Austria) ranged from 0 to 85 % Brix. The digital refractometer was calibrated with distilled water before each set of analyses.

3.4 Radial immunodiffusion

All samples were thawed at room temperature and thoroughly inverted by hand before RID analysis. A ready-to-use kit was used (IgGBov1, IDBiotech, Issoire, France) according to the manufacturers` instructions. The kit used horse anti-bovine IgG1. In brief, 500 µl of colostrum were diluted (1:100 with 0.15M PBS followed by 1:10 with SRID buffer; provided by the manufacturer) and pipetted into each well of a bovine IgG RID test plate. The plates were incubated for 18-24h at 37°C. The diameters of the ring precipitates were measured visually using the IDRing® Viewer-S (IDBiotech, Issoire, France). A linear calibration curve was established by the IDRing® Meter Software (IDBiotech, Issoire, France) according to the standards. Correlation coefficients of the standard curve had to be equal or higher 0.99 to be accepted, otherwise measurements were repeated. Samples were compared with the standard curve to determine IgG1 concentration. Samples outside the standard curve were retested at a different dilution. All samples were tested in duplicates and
only duplicate samples that did agree within the mean values (mean) ± 10% standard deviation (SD) were approved.

3.5 Statistics

Data were statistically analysed using PASW, version 20.0 (IBM Cooperation, New York, USA). The level of significance for all statistical tests was set at P < 0.05. To determine inter- and intra-rater reliability for both Brix refractometers, independent measurements were compared by Spearman correlation coefficient and $R^2$ statistics. Furthermore, results were categorized according to the previously reported cut-offs of 21, 22 and 23 % Brix [17-19] to calculate Cohen’s kappa (WATSON and PETRIE, 2010).

For RID, hydrometer 1 and 2, optical and digital Brix refractometer descriptive statistics (mean, SD, minimum, maximum, and percentage of samples up to and over a given cut-off) were calculated. Based on IgG concentrations determined by RID, samples were classified as ‘poor’ (≤ 50 g/L) and ‘good’ (> 50 g/L) quality colostrum. Results of the measurements obtained by hydrometer 1 and 2 were classified as ‘poor’ and ‘good’ quality according to the cut-offs given by the manufacturer as 1047 and 1045, respectively. For the optical and digital Brix refractometer three cut-offs of 21, 22 and 23 % as reported in literature were used (BIELMANN et al., 2010; QUIGLEY et al., 2013; BARTIER et al., 2015). Based on this classification, sensitivity and specificity, as well as negative and positive predictive values (NPV and PPV) were calculated with RID results as reference. Sensitivity and specificity were defined as the probability to correctly classify colostrum of ‘poor’ and ‘good’ quality, respectively compared with the results of the RID. To identify optimal cut-offs to distinguish between ‘poor’ and ‘good’ quality colostrum, receiver operating characteristic (ROC) analyses were performed. As part of this analyzes the true positive rate (sensitivity) was plotted against the false positive rate (1-specificity) for the complete range of cut-off points for each device. The area under the ROC curve (AUC) was used to rate the quality of the analysed cut-offs (SWETS, 1988).
The correlations between the reference method concentrations of IgG and the results of the four different devices were calculated as Spearman correlation coefficients. R² statistics were calculated for all devices.
4 Results

4.5 Pre-study

One and four of the 150 samples tested for inter- and intra-rater reliability, respectively, could not be evaluated. One sample could not be homogenized satisfactorily and in four samples no definite blue line appeared on the scale of the optical Brix refractometer. Spearman correlation coefficient calculated for the measurements performed by two independent observers was 0.97 and $R^2$ was 0.96 (Figure 1). Correlation between results of two independent measurements by the same observer using the optical and digital Brix refractometer was 0.99 and 0.98. Cohen’s kappa was 0.90 for the measurements by two independent observer. For the repeated measurements by one observer with the optical and digital refractometer, the Cohen’s kappa was 0.90 and 0.92.

4.6 Descriptive statistics

Two of the 195 samples were excluded from the study due to insufficient volume for hydrometer testing. IgG concentrations obtained by RID ranged from 5.8 to 168.7 g/L and showed a normal distribution (Figure 2). Based on RID results, 34.7 % of the samples were classified as ‘poor’ quality ($\leq$ 50 g/L), 65.3 % as ‘good’ quality (> 50 g/L) colostrum (Table 1).

4.7 Test characteristics

Sensitivity and specificity with reported cut-offs were calculated and are shown in Table 2. Sensitivity ranged from 90 to 96 % for all devices. Specificities were 34 and 27 % for hydrometer 1 and 2, and 31 and 47 % for the optical and digital Brix refractometer. Cut-offs for balanced sensitivity and specificity evaluated by ROC analysis were larger for all devices than the reported cut-offs (Table 2). The AUC calculated for each of the four devices differed significantly from the area under the
major diagonal line (AUC = 0.5; P < 0.01). The AUC of hydrometer 2 was significantly (P < 0.05) smaller than the AUC of the other three devices. The AUC of hydrometer 1, and the two Brix refractometers did not differ significantly from each other (P = 0.38-0.99). The ROC curves for all devices and RID are shown in Figure 3.

4.8 Correlation

Spearman correlation coefficients between the reference method RID and the different devices are presented in Table 3. Lowest coefficient was calculated for hydrometer 2 with 0.49 and highest for the digital Brix refractometer with 0.68. R² for hydrometer 1 and 2 were 0.43 and 0.26, and for the optical and digital Brix refractometer 0.40 and 0.47.
Correlations between the four devices and RID for samples with IgG ≤ 50 g/L and samples > 50 g/L are presented in Table 4.
5 Discussion

Precision evaluates the consistency of the measuring process in terms of repeatability and reproducibility (VIERA and GARRETT, 2005). High inter-observer agreement using a hydrometer already has been reported ($r=0.98$) (MECHOR et al., 1991). But so far, to our knowledge, no data concerning repeatability and reproducibility of estimating colostrum quality by Brix refractometers are available. Consequently, two independent measurements of one sample by the same observer with the optical and digital Brix refractometer were performed. The calculated Spearman correlation coefficient and Cohen’s kappa indicated an almost perfect agreement for both refractometers. Correlation and Cohen’s kappa between the two observer-measurements were equivalent. These results indicate excellent precision in terms of repeatability and reproducibility (Figure 3).

The main objective of the present study was the evaluation of two hydrometers and two Brix refractometers in order to determine IgG content in first colostrum on farm. As widely recognized, RID is the reference method for IgG concentration measurement (OYENIYI and HUNTER, 1978; FLEENOR and STOTT, 1980).

In the present study, the mean IgG concentration was lower and the proportion of ‘poor’ quality samples ($\leq 50$ g IgG/L) was greater compared to other studies evaluating devices in the field (Table 5). Hence, the current study represents a wider distribution of IgG concentrations.

Several factors, however, may have caused the differences in IgG concentrations among the reported studies e.g. breed, timing of colostrum collection, parity, month of calving, nutrition, and environment (PRITCHETT et al., 1991; TYLER et al., 1999; HOSTETLER et al., 2003; MCGUIRK and COLLINS, 2004; MOORE et al., 2005; GULLIKSEN et al., 2008; CONNEELY et al., 2013).

Correlation coefficients of the two hydrometers and RID differed considerably between the two different devices used. Consequently, it is not possible to provide generally valid recommendations regarding the use of a hydrometer on farm. Other studies examining hydrometers described correlations of 0.63 to 0.87 (MECHOR et al., 1992; QUIGLEY et al., 1994). These results were similar or higher compared with
hydrometer 1 of the present study and generally higher compared with hydrometer 2 of our study. Both hydrometers revealed high sensitivity of 94 and 90 % and low specificity of 34 and 27 % at the cut-offs provided by the manufacturer. Considering the results, most calves will be provided with ‘good’ colostrum quality. But two of every three ‘good’ quality colostrum sample would be falsely classified as not acceptable which may result in discarding a considerable amount of colostrum of ‘good’ quality.

Regarding test characteristics at Brix percentages of 21, 22, and 23 % as reported in literature (BIELMANN et al., 2010; QUIGLEY et al., 2013; BARTIER et al., 2015), for both refractometers best results were obtained at the highest cut-offs. In contrast to the other devices, in the present study the digital Brix refractometer showed best test characteristics, but overall lower than in other studies (BIELMANN et al., 2010; QUIGLEY et al., 2013; VANDEPUTTE et al., 2014). Highest accuracy was at 27 and 23.5 % for optical and digital Brix refractometer as determined by ROC analysis. The difference between the balanced cut-off of the optical Brix refractometer compared with other studies may be affected by the occurrence of a wide band formation on the Brix scale for some samples, which complicated interpretation. A balanced Brix cut-off for the digital Brix refractometer, however, was similar to findings of CHIGERWE et al. (2008) and BARTIER et al. (2015), but higher compared with 21 to 22.0 % as reported by others (VANDEPUTTE et al., 2014).

Likewise, correlation coefficients for Brix refractometers against RID were similar to the reports of CHIGERWE et al. (2008) and BARTIER et al. (2015), but lower than reported by others (BIELMANN et al., 2010; QUIGLEY et al., 2013). Factors such as the use of different correlation coefficients (Spearman correlation coefficient versus Pearson correlation coefficient) and different immunoglobulin classes (IgG1 versus IgG) have to be considered when comparing the results with published data. A potential reason for lower correlation between the Brix refractometers and the RID in the present study compared with literature (BIELMANN et al., 2010; QUIGLEY et al., 2013; VANDEPUTTE et al., 2014) could be due to the different proportions of ‘poor’ and ‘good’ quality colostrum samples in our study. Studies with better test results included mainly ‘good’ quality colostrum samples (84 to 92 %) (BIELMANN et
al., 2010; QUIGLEY et al., 2013; VANDEPUTTE et al., 2014), whereas in our study and studies with similar results the proportion of ‘poor’ quality samples ranged between 29 and 35 % (CHIGERWE et al., 2008; BARTIER et al., 2015). To support this hypothesis, we examined ‘poor’ (≤ 50 g IgG/l) and ‘good’ (> 50 g/L) quality colostrum samples separately. Correlation between Brix refractometers and RID results were better for samples above compared with samples equally or below the cut-off (50 g/L). Based on these findings, it is likely that the level of IgG concentration is associated with the degree of correlation.

Additionally, parity may have influenced the test results. BIELMANN et al. (2010) distinguished between parities and described different correlation coefficients for the digital Brix refractometer compared with RID. According to their study, strongest correlation was found for first lactation cows, lowest for second lactation cows. As in the present study only cows in 2nd and higher parity could be examined, this may be another explanation for lower correlations obtained.

Recent studies reported that colostrum quality is rarely controlled on dairy farms (GODDEN, 2008; VASSEUR et al., 2010; KLEIN-JÖBSTL et al., 2014; STANEK et al., 2014). According to producers´ statements, the most common method is the use of a hydrometer. Therefore we included hydrometer testing in our study. As shown, some hydrometers may provide acceptable measurements, but still some disadvantages have to be considered. As well known, they are temperature sensitive, and compared with the use of the Brix refractometer relative large amounts of colostrum are needed for testing. Furthermore, hydrometers are fragile, what seems to be inconvenient for on farm use.

Referring to the results, the digital Brix refractometer showed appropriate test characteristics and provided most accurate measurement of all evaluated devices. It is an inexpensive, easy to use, on farm device that can be used temperature independent. In contrast to the optical Brix refractometer, the result is presented in digital numbers on screen which leaves no room for misinterpretation. Additionally, the intra-rater reliability was almost perfect, indicating excellent precision in terms of repeatability. In the present study the optimal cut-off for the digital Brix refractometer
of 23.4% Brix was similar to cut-offs recommended by CHIGERWE et al. (1999) and BARTIER et al. (2015).

The digital Brix refractometer seems currently to be the best available method for assessing colostrum quality on farm.
6 Conclusion

An adequate quantity of IgG in colostrum is necessary to ensure optimal health for newborn calves. To improve colostrum management and to encourage more producers to evaluate colostrum quality, a practicable on farm device is needed. This study showed that hydrometers can be appropriate devices, but they vary widely depending on the type. Brix refractometers are suitable on farm tools that perform well at any temperature. The optimal cut-off was determined at 23.4%. The use of a digital Brix refractometer is currently the best available device to determine colostrum quality on farm.
7 Deutsche Zusammenfassung

Das Kolostrummanagement, insbesondere die Kontrolle der Kolostrumqualität spielen eine bedeutende Rolle in Milchviehbetrieben, um eine ausreichende passive Immunität von neugeborenen Kälbern zu gewährleisten. Um die Kolostrumqualität am Betrieb zu überprüfen, ist eine schnelle, kostengünstige und zuverlässige Methode notwendig.

Das Ziel dieser Studie war es, zwei unterschiedliche Hydrometer, sowie ein optisches und ein digitales Brix Refraktometer im Vergleich zu den Ergebnissen der Referenzmethode radiale Immunodiffusion (RID), auf Genauigkeit und Präzision bei der Bestimmung der Qualität von Kolostrum zu testen.


Für die Hauptstudie wurden 193 Erstkolostrumproben von Holstein Kühen eines großen Milchviehbetriebes untersucht.

Als Schwellenwerte für die Hydrometer wurden die Herstellerangaben berücksichtigt. Für die Evaluierung der Brix Refraktometer wurden bereits publizierte Schwellenwerte herangezogen.

Die statistische Auswertung der Inter-, sowie Intrarater-Reliabilität ergab Spearman Korrelationskoeffizienten von ≥ 0,96 für beide Refraktometer, was auf eine exzellente Präzision hinweist. Von allen untersuchten Proben wiesen 34,9 % bei der RID eine Immunoglobulinkonzentration ≤ 50 g/L auf und wurden in weiterer Folge als „schlechte“ Qualität eingestuft. Verglichen mit den Ergebnissen der RID war der Spearman Korrelationskoeffizient für die beiden Hydrometer 0,65 und 0,49, sowie 0,62 und 0,68 für das optische und digitale Refraktometer. In weiteren Untersuchungen reichte die Korrelation für Kolostrum von „schlechter“ Qualität von 0,17 und 0,35 und war damit signifikant niedriger als die Korrelation für Kolostrum
von „guter“ Qualität (0,50-0,62). Dies lässt einen Zusammenhang zwischen dem Gehalt an Immunoglobulin G-Konzentration im Kolostrum und der Korrelation vermuten.

Wurden die vorgegebenen Schwellenwerte zur Beurteilung der Kolostrumqualität herangezogen, so lag die Sensitivität der vier untersuchten Geräte (zwei Hydrometer, optisches und digitales Brix Refraktometer) zwischen 0,90 und 0,96. Die ermittelte Spezifität war hingegen deutlich niedriger und reichte von 0,27 bis 0,47. In der vorliegenden Studie ergaben sich bei der Receiver Operating Curve (ROC) Analyse, beste Testcharakteristika bei Schwellenwerten von 1055 bzw. 1054 für die beiden Hydrometer und bei 27 bzw. 23,4 % Brix für das optische und digitale Brix Refraktometer. Diese Werte liegen für alle Geräte über den vom Hersteller bzw. in der Literatur angegebenen Werten.

Zusammenfassend und in Hinblick auf die jeweiligen Testeigenschaften, lässt sich festhalten, dass das Brix Refraktometer die derzeit beste Methode ist, um Kolostrumqualität am Betrieb zu überprüfen. Es ist einfach in der Handhabung, zuverlässig und liefert akkurate Resultate.
8 References


9 Tables

9.1 Table 1

Descriptive statistics of the four devices and the reference method radial immunodiffusion (RID) for 193 first colostrum samples.

<table>
<thead>
<tr>
<th>Method</th>
<th>RID (g/L)</th>
<th>Hydrometer 1&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Hydrometer 2&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Optical Brix refractometer (%)</th>
<th>Digital Brix refractometer (%)</th>
</tr>
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<tr>
<td>Mean</td>
<td>64.9</td>
<td>1057.5</td>
<td>1056.1</td>
<td>25.8</td>
<td>25.4</td>
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<td>SD&lt;sup&gt;2&lt;/sup&gt;</td>
<td>28.6</td>
<td>10.8</td>
<td>9.5</td>
<td>4.4</td>
<td>4.5</td>
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<tr>
<td>Minimum</td>
<td>6.0</td>
<td>1031.0</td>
<td>1032.0</td>
<td>13.0</td>
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<td>Maximum</td>
<td>168.6</td>
<td>1085.0</td>
<td>1080.0</td>
<td>380.0</td>
<td>40.1</td>
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<tr>
<td>Cut-off</td>
<td>50.0&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1047&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1045&lt;sup&gt;4&lt;/sup&gt;</td>
<td>21.0&lt;sup&gt;5&lt;/sup&gt;</td>
<td>22.0&lt;sup&gt;6&lt;/sup&gt;</td>
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<tr>
<td>%&lt;sup&gt;8&lt;/sup&gt; ≤ cut-off</td>
<td>34.7</td>
<td>15.5</td>
<td>15.5</td>
<td>9.8</td>
<td>13.5</td>
</tr>
<tr>
<td>%&lt;sup&gt;8&lt;/sup&gt; &gt; cut-off</td>
<td>65.3</td>
<td>84.5</td>
<td>84.5</td>
<td>90.2</td>
<td>86.5</td>
</tr>
</tbody>
</table>

1 Specific gravity
2 SD = standard deviation
3 According to Godden et al. (2008)
4 According to the manufacturer
5 According to Quigley et al. (2013)
6 According to Bielmann et al. (2010)
7 According to Bartier et al. (2015)
8 % of samples
### 9.2 Table 2

Test characteristics for all devices compared with the reference method radial immunodiffusion (RID) for 193 first colostrum samples

<table>
<thead>
<tr>
<th>Method</th>
<th>Hydrometer 1</th>
<th>Hydrometer 2</th>
<th>Optical Brix refractometer</th>
<th>Digital Brix refractometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut-off</td>
<td>1047(^1)</td>
<td>1045(^1)</td>
<td>21.0 22.0 23.0</td>
<td>21.0 22.0 23.0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.94</td>
<td>0.90</td>
<td>0.99 0.96 0.90</td>
<td>0.90 0.94 0.83</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.34</td>
<td>0.27</td>
<td>0.27 0.31 0.48</td>
<td>0.27 0.47 0.57</td>
</tr>
<tr>
<td>PPV(^5)</td>
<td>0.73</td>
<td>0.70</td>
<td>0.72 0.72 0.76</td>
<td>0.70 0.77 0.78</td>
</tr>
<tr>
<td>NPV(^6)</td>
<td>0.74</td>
<td>0.58</td>
<td>0.95 0.81 0.71</td>
<td>0.60 0.80 0.64</td>
</tr>
<tr>
<td>Cohen´s Kappa</td>
<td>0.33</td>
<td>0.20</td>
<td>0.31 0.32 0.41</td>
<td>0.34 0.45 0.41</td>
</tr>
<tr>
<td>AUC(^7)</td>
<td>0.79</td>
<td>0.71</td>
<td>0.79</td>
<td>0.81(^)</td>
</tr>
<tr>
<td>(95% CI)(^9)</td>
<td>(0.73-0.86)</td>
<td>(0.63-0.78)</td>
<td>(0.73-0.86)</td>
<td>(0.75-0.87)(^)</td>
</tr>
<tr>
<td>Optimized device cut-off(^8)</td>
<td>1055</td>
<td>1054</td>
<td>27</td>
<td>23.4(^)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.73</td>
<td>0.71</td>
<td>0.56</td>
<td>0.79(^)</td>
</tr>
<tr>
<td>(95% CI)(^9)</td>
<td>(0.64-0.81)</td>
<td>(0.62-0.78)</td>
<td>(0.47-0.65)</td>
<td>(0.71-0.86)(^)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.72</td>
<td>0.61</td>
<td>0.90</td>
<td>0.69(^)</td>
</tr>
<tr>
<td>(95% CI)(^9)</td>
<td>(0.59-0.82)</td>
<td>(0.56-0.79)</td>
<td>(0.80-0.96)</td>
<td>(0.56-0.79)(^)</td>
</tr>
</tbody>
</table>

---

\(^1\) According to the manufacturer
\(^2\) According to Quigely et al. (2013)
\(^3\) According to Bielmann et al. (2010)
\(^4\) According to Bartier et al. (2015)
\(^5\) PPV = positive predictive value
\(^6\) NPV = negative predictive value
\(^7\) AUC = area under the receiver operating characteristics (ROC) curve
\(^8\) Based on ROC analyses
\(^9\) 95 % confidence interval
### 9.3 Table 3

Spearman correlation coefficients for all tested devices and the reference method radial immunodiffusion (RID) for 193 first colostrum samples.

<table>
<thead>
<tr>
<th>Method</th>
<th>RID</th>
<th>Hydrometer 1</th>
<th>Hydrometer 2</th>
<th>Optical Brix refractometer</th>
<th>Digital Brix refractometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>RID</td>
<td>1.00</td>
<td>0.65</td>
<td>0.49</td>
<td>0.64</td>
<td>0.68</td>
</tr>
<tr>
<td>Hydrometer 1</td>
<td>0.65</td>
<td>1.00</td>
<td>0.70</td>
<td>0.65</td>
<td>0.78</td>
</tr>
<tr>
<td>Hydrometer 2</td>
<td>0.49</td>
<td>0.70</td>
<td>1.00</td>
<td>0.56</td>
<td>0.64</td>
</tr>
<tr>
<td>Optical Brix refractometer</td>
<td>0.64</td>
<td>0.65</td>
<td>0.56</td>
<td>1.00</td>
<td>0.91</td>
</tr>
<tr>
<td>Digital Brix refractometer</td>
<td>0.68</td>
<td>0.78</td>
<td>0.64</td>
<td>0.91</td>
<td>1.00</td>
</tr>
</tbody>
</table>

P for all correlation coefficients < 0.01
9.4 Table 4

Spearman correlation coefficient for samples classified as ‘poor' and ‘good' colostrum quality (≤ and > 50 g IgG/L colostrum) by the reference method radial immunodiffusion (RID), separately.

<table>
<thead>
<tr>
<th></th>
<th>RID ≤ 50 g/L</th>
<th></th>
<th>RID &gt; 50 g/L</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman correlation coefficient</td>
<td>P</td>
<td>Spearman correlation coefficient</td>
<td>P</td>
</tr>
<tr>
<td>Hydrometer 1</td>
<td>0.17</td>
<td>0.16</td>
<td>0.62</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Hydrometer 2</td>
<td>0.10</td>
<td>0.40</td>
<td>0.50</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Optical Brix</td>
<td>0.28</td>
<td>0.02</td>
<td>0.52</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>refractometer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digital Brix</td>
<td>0.35</td>
<td>&lt; 0.01</td>
<td>0.61</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>refractometer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9.5 Table 5
Published IgG concentrations and Spearman correlation coefficients with radial immunodiffusion (RID) as reference method

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Mean±SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>≤ 50</th>
<th>&gt; 50</th>
<th>Hydrometer 1</th>
<th>Hydrometer 2</th>
<th>Optical Brix refractometer</th>
<th>Digital Brix refractometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>195</td>
<td>64.9±28.6</td>
<td>5</td>
<td>168.7</td>
<td>35</td>
<td>65</td>
<td>0.65</td>
<td>0.49</td>
<td>0.61</td>
<td>0.67</td>
</tr>
<tr>
<td>Bartier et al. (2015)</td>
<td>460</td>
<td>63.7±2.2</td>
<td>8</td>
<td>128.6</td>
<td>29</td>
<td>71</td>
<td>0.77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vandeputte et al. (2014)</td>
<td></td>
<td>95.5±36.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quigley et al. (2013)</td>
<td>183</td>
<td>73.4±26.2</td>
<td>7</td>
<td>159.0</td>
<td>16</td>
<td>84</td>
<td></td>
<td>0.75</td>
<td></td>
<td>0.64</td>
</tr>
<tr>
<td>Bielmann et al. (2010)</td>
<td>273</td>
<td>94.4</td>
<td>22</td>
<td>196.9</td>
<td>8</td>
<td>92</td>
<td></td>
<td></td>
<td>0.71</td>
<td>0.73</td>
</tr>
<tr>
<td>Chigerwe et al. (2008)</td>
<td>171</td>
<td>68.5±32.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quigley et al. (1994)</td>
<td></td>
<td>69.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechor et al. (1992)</td>
<td>39</td>
<td>37±13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Beef cow colostrum samples
2 Pearson correlation coefficient
3 Cut-off for ‘poor’ and ‘good’ quality were set at < 50 an ≥ 50 g/L
4 Differentiation between parities
   - 1. lactation 0.77/0.83
   - 2. lactation 0.68/0.71
   - ≥ 3. lactation 0.71/0.73
10 Figures

10.1 Figure 1

Figure 1. Relationship between independent measurements (n = 146) by two observers with the optical Brix refractometer.
10.2 Figure 2

Figure 2. Distribution of immunoglobulin G (IgG) concentration obtained by the reference method radial immunodiffusion (RID) for 193 first colostrum samples. The black line symbolizes the cut-off of > 50.0 g/L IgG for ‘poor’ (left) and ‘good’ (right) quality colostrum.
Figure 3. Receiver operating characteristics (ROC) curve for the four tested devices and radial immunodiffusion (RID) as the reference method.